1073

ACIDIC AND NEUTRAL CARBONYL COMPOUNDS IN SWISS CHEESE (1) (2)

E. W. BASSETT and W. J. HARPER
The Ohio Agricultural Experiment Station and The Ohio State University, Department of Dairy Technology

With recent advances in microanalytical techniques, such as chromotography, electrophoresis and radioautography, considerable progress has been made to obtain a better understanding of the complex cheese ripening process. In Swiss cheese investigators have emphasized either amino acids or fatty acids formed during ripening and propionic acid, acetic acid, proline, glutamic acid have been found to be important end products. (4) (7) (12) (14)

Hintz (7) showed that the concentration of certain amino acids in Swiss cheese first increased and then decreased during the ripening period. This same observation was made by Herner and Tuckey (8) for Cheddar cheese and by Long (13) for Italian cheese varieties. A similar trend for acetic acid in Swiss cheese was by found by Slatter (14). These findings suggest that in addition to being important end-products, certain of the free aliphatic acids and amino acids may be important as precursors of other products. In this study attention was given to the acidic and neutral carbonyl compounds of Swiss cheese as being degradation and intermediate products of the various organic acids.

Procedure

The cheese samples were prepared for analysis by making a water extract as outlined by Sommer and Harper (14). Separation and

(1) Technical Paper 1:56 Department of Dairy Technology, The Ohio State University. Ohio Agriculture Experiment Station Journal Article.
(2) Supported in part by Research and Marketing Act of 1946, through the Dairy Technology Section, A. R. S., U. S. Department of Agriculture and the Ohio Dairy Products Research Fund.

identication of the carbonyl compounds in cheese is explained in detail in another paper (1).

The water extract was treated as follows: (a) 10 per cent sodium tungstate was added to precipitate the proteins; (b) the sample was chilled to 4°C to prevent destruction of beta-keto-acids and then adjusted to pH 4°C with 0.67 N sulfuric acid; (c) the water extract was filtered by suction through a very thin (1 mm) layer of silic acid (chromatographic grade); (d) the clear extract was reacted for one hour at 25°C with an excess of saturated 2.4 dinitrophenylhydrazine in 2-N hydrochloric acid; (e) the solution was extracted with ether, which was evaporated in a desiccator under vacuum at room temperature and finally by drying over calcium chloride and potassium hydroxide for twelve hours.

The dry hydrazones were dissolved in a minimum of 1-N-ammonium hydroxide and washed with sufficient chloroform to remove the neutral carbonyls and hydrazine. The acidic compounds were streaked in a narrow band on Whatman No. 3 MM filter paper. After the band was dry, the chromatogram was developed according to the method of El-Harway and Thompson (5), using butanol' 0.5 N-ammonium hydroxide and ethanol (70-20-10 v/v).

The chloroform, containing the neutral carbonyls, was evaporated to dryness and the residue was dissolved in a small quantity of benzene-heptane (1-1). The residual hydrazine was removed by passing the solution through an anhydrous magnesium sulfate column. The chromatographic method of Heulin (6) was used for the separation of the neutral carbonyl compounds.

Results and discussion

Ten samples of domestic Swiss cheese, varying in age from two to eighteen months were analyzed for acids and neutral carbonyl compounds. The compounds isolated from each cheese were identified on the basis of the Rf value, color of the hydrazine derivative and light absorption characteristics of the derivatives as reported previously for compounds found in cheese (1). Compounds were also identified by melting point determination when sufficient material was isolated. In all of the Swiss cheese samples examined in this study, the keto acid pattern remained essentially the same, regardless of age or flavor intensity of the cheese. However, the carbonyl pattern was affected when there was absence of eye formation. The carbonyl pattern found in typical Swiss cheese and « blind » Swiss is shown in Table 1. In typical Swiss cheese, about 80 per cent of the total carbonyls was found to be pyruvic acid (varying from 0.1 to 0.5 mg/g cheese) and about 10 per cent was alpha keto glutaric acid. Definite concentrations of alpha-acetolatic acid [CH_3 COH ($COCH_3$) COOH] and traces of oxalsuccinic acid, oxalacetic acid, diacetyl and acetylmethyl carbinol were found. The neutral carbonyl compounds were reported previously in Swiss cheese by Calbert and Price (2). In the « blind » or eyeless Swiss cheese the same compounds were found as in the normal cheese, but the proportion of pyruvic acid and alpha ketoglutaric acid were different. About 40 per cent of the total carbonyl compounds was pyruvic and about 40 per cent was alpha keto glutaric acid.

TABLE 1. — CARBONYL COMPOUNDS IN SWISS CHEESE

Carbonyl Compounds	Concentration of Compounds in (a)	
	Typical Swiss	« Blind » Swiss
oxalsuccinic acid	trace	trace
alpha-ketoglutaric acid	2	3
oxalacetic acid	trace	trace
Pyruvic acid (b)	4	3
alpha-acetolactic acid	1	1
acetyl methylcarbinol	trace	trace
diacetyl	trace	trace

(a) Relative concentration from 1 to 4; 1 = less than 10 per cent of total hydrazones, 2 = 10 to 30 per cent of total hydrazones; 3 = 40 to 70 per cent of total hydrazones, 4 = more than 70 per cent of total hydrazones.
(b) Present in two isomeric forms of hydrazone.

All of the compounds identified, especially pyruvic and alpha-keto-glutaric acids, are generally considered to be readily utilizable intermediate products. The presence of relatively high concentrations of pyruvic acid may indicate an important characteristic of the ripening process of the Swiss cheese.

In other studies conducted on milk cultures, the Swiss cheese pattern of keto acids was nearly duplicated. In milk culture of

Streptococcus thermophilus of *Lactobacillus bulgaricus,* it was found that the only difference between the culture pattern and that of Swiss cheese was the disproportionately large concentration of the Alpa-keto-glutaric acid in the cultures. However, when *Propionibacter shermanii* bacteria were included in the cultures, the resulting carbonyl pattern exactly duplicated that of Swiss cheese.

The most unusual compound identified in Swiss cheese was alpht-acetolactic acid. This compound is of especial interest in dairy products since it has been suggested as the logical immediate precursor of acetylmethyl carbinol and diacetyl (9) (10). It is extremely heat and acid labile, which probably explains why it had not been isolated previously.

A brief study was made to determine if acetolactic acid was an intermediate in the formation of acetylmethyl carbinol and diacetyl in Swiss cheese. Carbon-14 labeled sodium citrate was added to a freshly prepared water extract of Swiss cheese and to cultures of *L. bulgaricus.* The results with both were the same A typical chromatrogram and radioautograph are shown in Figure 1. The radioautograph shows that addition of C^{14} labeled citrate resulted in radioactive alpha-keto-glutarate, alpha acetolactate and the neutral carbonyls. It was interesting to note that the pyruvic acid formed was not C^{14} labeled. About 20 per cent of the C^{14} was in the alpha keto glutaric acid, 10 per cent in acetolactate and about 10 per cent in acetylmethol carbinol and diacetyl.

Further studies were made with sodium acetate-1-C^{14}, sodium malate-C^{14} and sodium succinate-1, 4 C^{14}. With acetate, only about 2 per cent of the C^{14} appeared in the carbonyl compounds, and this was found in acetolactate, acetylmethylcarbinol, and diacetyl. The C^{14} from malate appeared only in pyruvic acid. Carbon ^{14} from succinate appeared in the acetolactate and neutral carbonyl fractions. Further experiments are in progress to complete the study of the pathway of diacetyl formation.

In the course of this study, it was observed that the addition of alpha keto glutaric acid to Swiss cheese resulted in its rapid disappearance. Use of C^{14} citrate showed, however, that the alpha keto-glutaric acid was not the precursor of the pyruvic acid. This suggests the possible occurrance of transamination as an important reaction in the ripening of Swiss cheese.

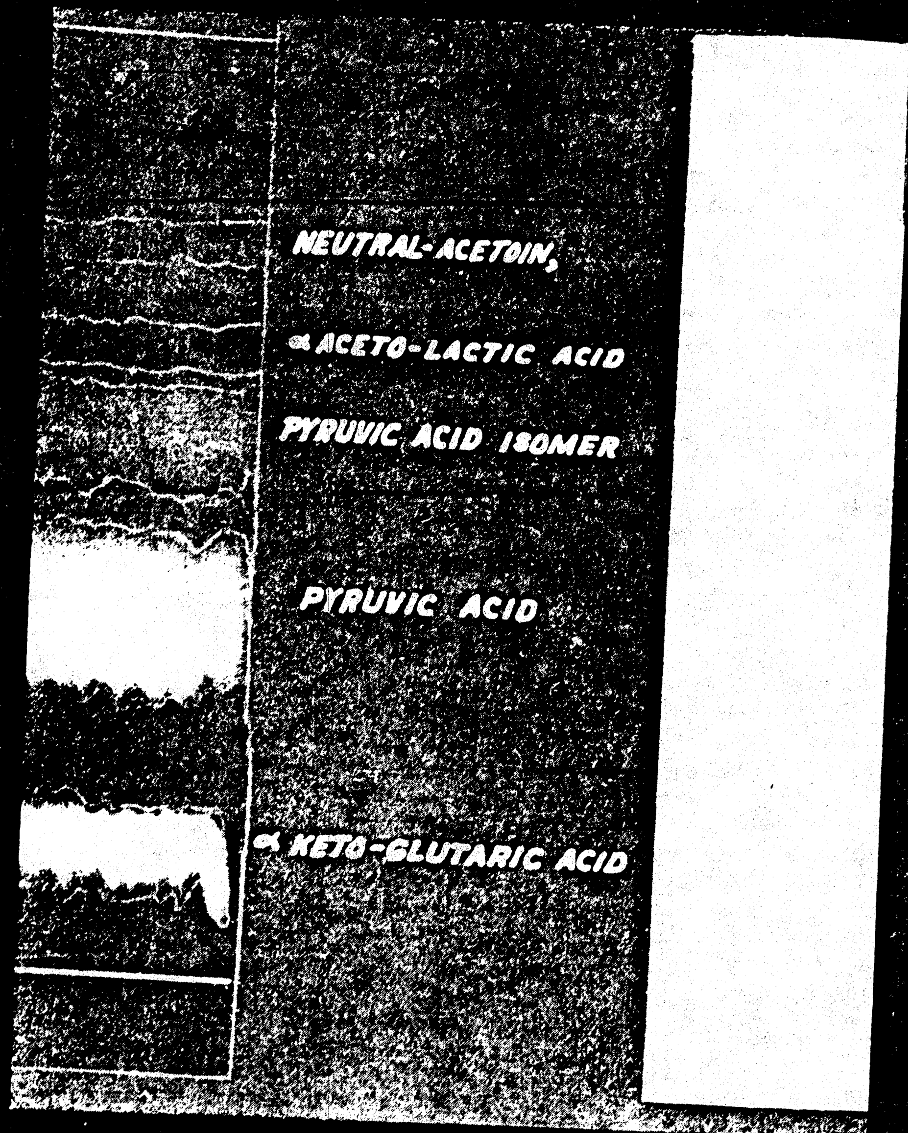

Fig. 1. — The Effect of adding C^{14} sodium citrate to *Lactobacillus bulgaricus* on the acidic and neutral carbonyl compounds. Chromatogram on left shows the keto compounds found in the culture after 12 hr. incubation at 40°C; radio-autogram on the right shows the keto compounds which contained C^{14}. It was obtained by exposing chromatogram on an X-ray plate for 14 days.

REFERENCES

(1) BASSETT E. W. and HARPER W. J.: *Separation and Identification of Acid and Neutral Carbonyl Compound in Cheese.* In press 1956.

(2) CALBERT H. E. and PRICE W. V.: *Study of the Diacetyl in Cheese. I. Diacetyl Content and Flavor of Cheddar Cheese.* J. Dairy Sci. 32: 515-520 1949.

(3) CAVALLINI D. and FRONTALI N.: *Quantitative Determination of Keto Acids by Paper Partition Chromatography.* Biochem, et Biophys.

(4) DEMETER K. J.: *Bestimmung der Propionalsaure in der Entwicklung des Aroma's von Emmentalen Kase.* Suddent Molkerei-Ltg. 70: 862-3 1949.

(5) EL HANWARY M.F.S. and THOMPSON R. H.: *Estimation of Blood Keto Acids.* Biochem. J. 53: 340-47 1953.

(6) HEULIN E. F.: *Volatile Products of Apples. III Identification of Aldehydes and Ketones.* Aust. J. Sci. Res. Series B 5: 328 1952.

(7) HINTZ P. C.: *A Study of Various Free Amino and Free Fatty Acids Found in Swiss Cheese. M. S. Thesis.* The Ohio State University 1953.

(8) HORNER J. C. and TUCKEY S. L.: *Procedure for Quantitative Determination of Amino Acids by Paper Chromatography.* J. Dairy Sci. 34: 475 (Abst.) 1953.

(9) JUNI E.: *Mechanism of the Formation of Acetoin by Bacterial Synthesis.* J. Biol. Chem. 195: 715-26 1952.

(10) KOBAYASH Y. and KALNITSKY Y.: *The Bacterial Synthesis of Alpha Acetolactate.* J. Biol. Chem. 211: 473-82 1954.

(11) KOSIKOWSKY F. V.: *Liberation of Free Amino Acids in Raw and Pasteurized Milk Cheddar Cheese During Ripening.* J. Dairy Sci.

(12) KRETT O. J. and STINE J. B.: *The Role of Lower Fatty Acids in Swiss Cheese.* J. Dairy Sci. 34: 476 (Abst.) 1951.

(13) LONG J. E.: *The Relationship of Free Amino Acids to the Ripening of Italian Cheese.* The Ohio State University 1952.

(14) SLATTER W. L.: *Unpublished Data* Ohio State University 1953.

(15) SOMMER H. H. and HARPER W. J.: *Methods for Studying the Ripening of Cheese.* J. Dairy 31: 714-15 (Abst.) 1948.

SUMMARY

In addition to acetylmethylcarbinol and diacetyl, a group of acidic carbonyl compounds were found in Swiss cheese. Pyruvic acid, alpha keto-glutaric acid, acetolatic acid, oxalsuccinic acid and oxalacetic acid were present in all cheeses. The formation of eyes in the cheese was shown to be related to the keto acid pattern of the cheese.

Acetolactic acid was shown to be an intermediate in the conversion of citrate to diacetyl. Radioactive isotope experiments

were presented as a means to elucidate the pathway of diacetyl production in Swiss cheese and in lactic acid-producing starter cultures.

RESUME

LES COMPOSES DE CARBONYL ACIDIC ET CARBONYL NEUTRAL DANS LE FROMAGE SUISSE

En plus de l'acetylméthylcarbinol et du diacétyl, l'on a trouvé dans le fromage suisse un groupe de composés de carbonyl acidic. L'acide pyruvic, l'acide alpha keto-glutario, l'acide acétolactique, l'acide oxalsuccinic et l'acide oxalacetic furent trouvés dans tous les fromages.

Le spécimen d'acide kétonique du fromage provoque la formation des yeux dans ce dernier.

Il fut démontré que l'acide acétolactique sert d'intermédiaire dans la conversion du citrate en diacétyl.

On a présenté les résultats des expériences isotopes radioactives comme moyen de mieux connaître le passage de la production de diacétyl dans le fromage suisse et dans l'acide lactique producteur de cultures de ferments.

ZUSAMMENFASSUNG

SAURE UND NEUTRALE CARBONYLVERBINDUNGEN IN SCHWEIZER KÄSE

Ausser Acetylmethylcarbinol und Diacetyl wurde in Schweizer Käse eine Gruppe von sauren Carbonylverbindungen gefunden. Pyruvin-, Alpha-Ketoglutarin-, Acetomilchsäure, Oxalsuccin- und Oxalessigsäure waren in allen Käsen vorhanden. Die Bildung von Löchern konnte auf den Bestand an Ketosäuren in dem betreffenden Käse zurückgeführt werden. Acetomilchsäure wurde als eine Zwischenstufe beim Übergang von Zitrat in Diacetyl erkannt. Versuche mit radioaktiven Isotopen werden als ein Mittel dargestellt, um den Verlauf der Diecetylentstehung im Schweizer Käse und in Milchsäure bei der Bildung von Säureweckern zu verfolgen.